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SH-GROUPS ON THE SURFACE OF PANCREAS CELLS INVOLVED IN SECRETIN STIMULATION AND GLUCOSE-MEDIATED SECRETION

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SUMMARY

A large derivative of *p*-chloromercuribenzoic acid (PCMB–Dextran T 10, mol. wt approx. 10000) was perfused through an isolated preparation of cat pancreas. It was shown that PCMB–Dextran T 10 inhibited pancreatic fluid and electrolyte secretion by 47%. When glucose in the perfusate was replaced by other substrates, PCMB–Dextran T 10 caused an inhibition of only 26%. Replacement of secretin by dibutyryl cyclic AMP and theophylline led to an inhibition of 15%. When secretin as well as glucose was replaced by its substitutes, no inhibition of secretion could be achieved with PCMB–Dextran T 10. Considering the large molecular size of this mercurial compound it can be concluded that SH-groups on the surface of the pancreatic cells are essential both for the action of secretin and for the glucose-mediated secretion.

INTRODUCTION

The effect of chemical reagents on membranes depends on their ligand specificity and their ability to reach functional sites with which they might react[1]. Many studies in different tissues have been performed with small mercurial compounds such as *p*-chloromercuribenzoic acid (PCMB), which is known to react specifically in low concentrations with sulphydryl groups[2]. Since these small molecules can penetrate the cell membrane[3], they can affect membrane processes, such as hormonal interactions with membrane receptor sites, permeability, active ion transport and substrate uptake[3], as well as intracellular processes.

In order to determine whether or not SH-groups on the surface of the pancreas are involved in pancreatic fluid secretion, a large derivative of PCMB–aminoethyl-Dextran T 10, mol. wt approx. 10000 (PCMB–Dextran T 10) was perfused through an isolated preparation of cat pancreas.

It could be shown that PCMB–Dextran T 10 reversibly inhibits the action of secretin, the duodenal hormone which stimulates pancreatic fluid secretion. It was also found that PCMB–Dextran T 10 reversibly inhibits the effect of glucose on pancreatic secretion. With respect to the molecular weight of PCMB–Dextran T 10, these observations suggest that superficially located SH-groups are involved in secretin action and in glucose-mediated secretion

Abbreviation: PCMB, *p*-chloromercuribenzoic acid.

METHODS

Perfusion experiments

21 adult cats of both sexes were anaesthetised with pentobarbital (60 mg/kg body wt). The pancreas was surgically isolated according to the method of Case *et al.*[4]. All vessels leading to other organs were ligated. Perfusion was *via* the coeliac and superior mesenteric arteries, with venous drainage *via* the superior mesenteric vein. The main pancreatic duct was cannulated and secretion was measured in 10-min periods. A control period, which usually lasted 30–60 min, was followed by a period of 40–70 min in which PCMB–Dextran T 10 was added to the perfusate at a concentration of 10^{-8} M. A second control period followed, during which the perfusate contained 10^{-3} M cysteine. The composition of the perfusion fluid in mmoles/l was as follows: NaCl, 104; $NaHCO_3$, 26; KCl, 4.8; $MgCl_2$, 1.2; $CaCl_2$, 2.5; and D-glucose, 15. The pH of the perfusate was 7.4 and it was perfused through the pancreas at a temperature of 36 °C.

Synthesis of PCMB–Dextran T 10

Aminoethyl-Dextran T 10 was prepared from Dextran T 10 (mol. wt approx. 10^4) with 2-aminoethyl hydrogensulphate according to Eldjarn and Jellum[5]. The coupling of aminoethyl-Dextran T 10 to PCMB was performed by the method of Ohta *et al.*[6] with some major modifications which will be described in detail elsewhere (Simon, B., Zimmerschied, G., Kinne, R., unpublished). To characterize this new compound the glucose content of the Dextran T 10 was determined by the anthrone method[7] and amino groups with 2,4,6-trinitrobenzenesulphonic acid by the method of Habeeb[8]. Dr Herrmann from the analytical laboratory of the Farbwerke Hoechst, Frankfurt/M., kindly measured the mercury content by the dithizone method according to the prescription of the Trade Hygiene Institut, Norway. The results obtained showed the product to contain glucose units, amino groups and mercury in the following proportions: 50:3:1.

This particular Dextran T 10 was suitable for our purposes because of its high solubility, relative lack of toxicity and its molecular size. It is small enough to cross the capillaries but too large to penetrate cell membranes. The actions of PCMB and PCMB–Dextran T 10 on (Na^+–K^+)-ATPase activity from plasma membranes of rat kidney cortex yielded identical dose–inhibition curves (Simon, B., Zimmerschied, G., Kinne, R., unpublished), thus indicating that the linking of PCMB to Dextran T 10 does not influence the action of PCMB.

RESULTS

In the unstimulated cat pancreas preparation no basal volume output could be observed. Secretion could be stimulated, and the rate of flow maintained constant, by either the addition of secretin (0.1 unit/min) or of 10^{-3} M dibutyryl cyclic AMP *plus* $5 \cdot 10^{-3}$ M theophylline to the perfusate (Figs 1 and 2). This observation is consistent with the findings of Case *et al.*[9] that the secretin effect can be mimicked by dibutyryl cyclic AMP and theophylline. Glucose or other substrates are necessary to maintain pancreatic fluid secretion[10]. In 7 experiments we observed that the secretory rate was unchanged when the perfused glucose (15 mM) was replaced by α-ketoglutarate, fumarate and pyruvate (5 mM each). In order to differentiate the influence of

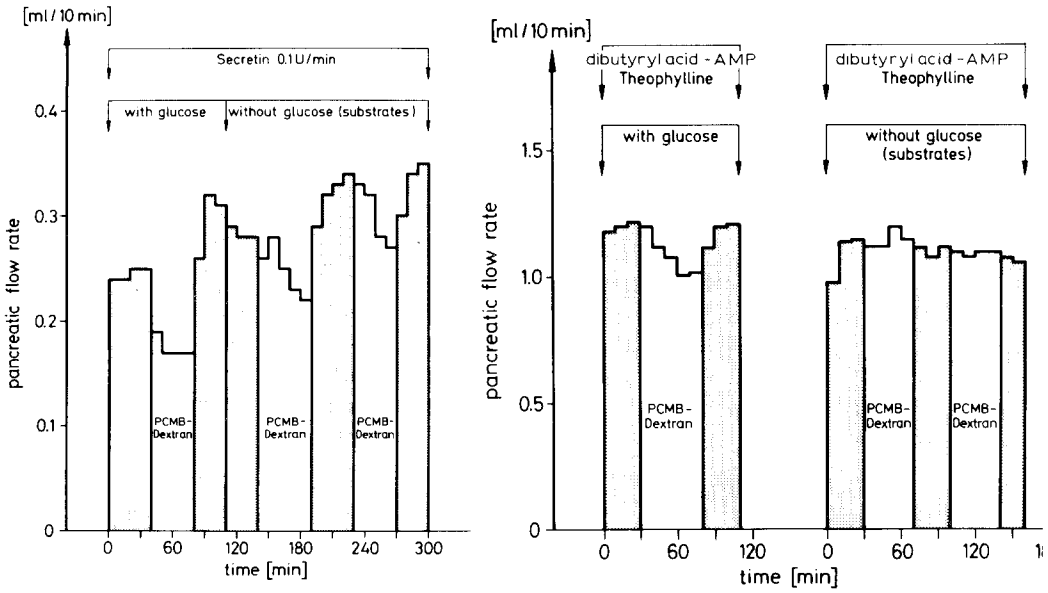

Fig. 1. Effect of PCMB-Dextran T 10 (10^{-8} M) on pancreatic secretion rate in the presence and absence of perfusate glucose during secretin stimulation. During the period following perfusion with PCMB-Dextran T 10, the perfusate contained cysteine (10^{-3}M).

Fig. 2. Effect of PCMB–Dextran T 10 (10^{-8} M) on pancreatic flow rate in the presence and absence of perfusate glucose during stimulation with 10^{-3} M dibutyryl cyclic AMP *plus* $5 \cdot 10^{-3}$ M theophylline. During the period following perfusion with PCMB–Dextran T 10, the perfusate contained cysteine (10^{-3} M).

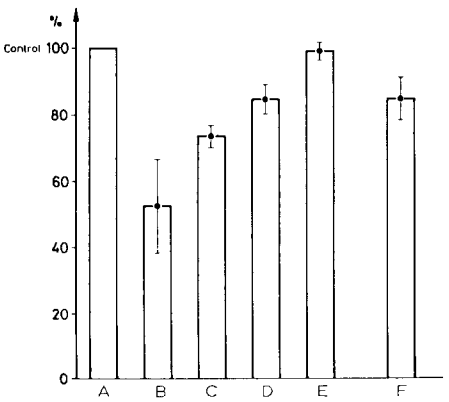

Fig. 3. Effect of PCMB–Dextran T 10 (10^{-8} M) on secretin (0.1 unit/min)-stimulated or dibutyryl cyclic AMP (10^{-3} M) *plus* theophylline ($5 \cdot 10^{-3}$ M)-stimulated pancreatic volume flow. The substrate was either glucose (15 mM) or α-ketoglutarate, fumarate and pyruvate (each 5 mM). Each set of experiments was done with paired controls (without PCMB–Dextran T 10). The means ± the S.D. of the means are given. A, control; B, PCMB–Dextran secretin glucose; C, PCMB–Dextran secretin substrates; D, PCMB–Dextran dibutyryl cyclic AMP *plus* theophylline glucose; E, PCMB–Dextran dibutyryl cyclic AMP *plus* theophylline substrates and; F, secretin glucose phloretin 10^{-5} M.

PCMB–Dextran T 10 on membrane processes involved in secretion, four experimental arrangements were used:

Stimulation with secretin; glucose as substrate (Figs 1 and 3)

The addition of PCMB–Dextran T 10 (10^{-8} M) to the perfusate inhibited pancreatic secretion by 47.3% (S.D. ± 14%, $n=6$, 4 experiments). The inhibition could be reversed by a perfusate containing 10^{-3} M cysteine.

This degree of inhibition should represent the overall effect of the SH-reagent at the plasma membrane of the pancreatic cell. Since it is known that PCMB–Dextran T 10 does not influence ion transport in the kidney[11], we neglected a possible effect on ion permeability and active ion transport (particularly the (Na^+–K^+)-ATPase activity[3]) and assumed that the total inhibition of 47% consists of two components: (a) Inhibition of the hormone (secretin) action and (b) inhibition of glucose-mediated secretion. To test this hypothesis, both substances, secretin and glucose, were substituted as follows:

Stimulation with dibutyryl cyclic AMP plus theophylline; "Krebs intermediates" as substrates (secretin and glucose replaced) (Figs 2 and 3)

Virtually no inhibition of pancreatic fluid secretion could be demonstrated under these conditions ($n=6$, 4 experiments). From these results it appears unlikely that, at the concentrations of PCMB–Dextran T 10 used, transport processes other than those considered are affected. In an attempt to confirm that the effect of glucose on secretion is one of the two processes which is inhibited by the SH-reagent, we modified our system as follows:

Stimulation with dibutyryl cyclic AMP+theophylline; glucose as substrate (secretin replaced) (Figs 2 and 3)

Under these conditions PCMB–Dextran T 10 reversibly inhibited secretion by only 15.4% (S. D. ± 4.3%, $n=5$, 4 experiments). In order to determine whether or not this small effect of PCMB–Dextran T 10 could be accounted for by the inhibition of glucose uptake, phloretin (10^{-5} M) was added to the glucose-containing perfusate. Under these conditions phloretin inhibited secretion by 13.8% (S.D. ± 6.4%, $n=6$, 5 experiments, Fig. 3). However, when "Krebs intermediates" instead of glucose were present in the perfusate, no inhibition could be achieved with 10^{-5} M phloretin. To test whether the secretin action is the other membrane process which is inhibited by PCMB–Dextran T 10, we provided the following experimental conditions:

Stimulation with secretin; "Krebs intermediates" as substrates (glucose replaced) (Figs 1 and 3)

When glucose was replaced by "Krebs intermediates" PCMB–Dextran T 10 (10^{-8}M) inhibited the secretin-stimulated secretion by 26.4% (S.D. ± 3.4%, $n=5$, 3 experiments). Using the plot of Fig. 3 it can be seen that the inhibitory effect of PCMB–Dextran T 10 on secretin action and on glucose-mediated secretion are additive.

DISCUSSION

It is probable that secretin, a polypeptide hormone of 27 amino acids, does not

enter the cell because of its large size but acts on the cell surface. The findings of Rutten *et al.*[12], that secretin stimulates a pancreatic adenylcyclase, and the experiments of Case *et al.*[9], showing a rise of pancreatic cyclic AMP content after administration of secretin, provide evidence that an adenylcyclase is involved in the "first hormonal target system" of secretin. Theophylline, an inhibitor of phosphodiesterase activity, prevents the breakdown of cyclic AMP. With both substances it is possible to mimick secretin action, thereby bypassing the adenyl cyclase step. Since secretin has no SH-groups, it is unlikely that secretin itself is affected by a SH-group reagent. One may therefore conclude that PCMB–Dextran T 10 with a molecular weight of about 10^4 interferes with SH-groups located at the interstitial surface of the pancreatic cell on which secretin acts. Using the same concentrations of PCMB–Dextran T 10 as in the perfusion experiments (10^{-8} M), we found a 50% inhibition of secretin-stimulated adenylate cyclase activity in a membrane fraction of the cat pancreas (Schulz, I. *et al.*, unpublished).

This finding, when taken together with the results of the perfusion experiments, indicates that highly reactive sulphydryl groups are involved in secretin action. Some hormone actions, which are mediated by cyclic AMP, are inhibited by SH-reagents[3]. In addition to our study, an action on adenylate cyclase activity has been shown only in the parotid gland[13], in the turkey erythrocyte[14] and in pig heart and lung[15]. It might therefore be possible that essential sulphydryl groups are the feature of all adenyl cyclases.

The molecular components of the pancreatic membrane, their geographic arrangement in the membrane structure and the sequence of changes that occur during the action of secretin are unknown. Thus, it cannot be decided whether PCMB–Dextran T 10 blocks sulphydryl groups associated to the active centre of the secretin receptor or neighbouring SH-groups, which secondarily influence the secretin receptor site.

It is known from many investigations that glucose uptake is inhibited by SH-group reagents (see ref. 3). Considering the equal inhibitory effect of phloretin, which specifically inhibits glucose uptake[15] and of PCMB–Dextran T 10 (Fig. 3), it can be assumed that it is the glucose uptake which is inhibited in our experiments. Concerning the superficial location of the sulphydryl groups affected by PCMB–Dextran T 10, a parallelism exists between our data and the findings of Vansteveninck *et al.*[17] that sugar transport in the erythrocyte membrane is controlled by a small population of SH-groups located near the outer surface. It should be noted that, at the concentrations of PCMB–Dextran T 10 used, only two of the membrane processes, secretin stimulation and glucose-mediated secretion are inhibited. PCMB–Dextran T 10, when added to the perfusate containing cysteine (10^{-3} M), had no influence on secretory flow rate when the pancreas was stimulated with secretin with glucose present as substrate. This further indicates that groups other than sulphydryl groups were not blocked by PCMB–Dextran T 10. The large molecular sulphydryl group reagent used in our experiments is of special value in determining the functional significance of specific groups on the membrane surface. It should be emphasized, however, that even when a membrane process involves SH groups, for example (Na^+–K^+)-ATPase, these SH groups may not be accessible to PCMB–Dextran T 10 *in vivo*. Similarly the glucose binding site in the renal brush border is sensitive to PCMB *in vitro* (Bode, F., Baumann, K., Frasch, W. and Kinne, R. (1969) *Pflügers Arch. Ges. Physiol.*

Menschen, Tiere 315, 53–65), but *in vivo* the renal glucose transport could not be inhibited by PCMB–Dextran. One may thus argue that the glycoprotein surface coat prevents the large SH reagent from reaching the functionally significant SH groups or that the isolation of the brush border induces configuration changes in the proteins altering the exposure of SH groups. Therefore, it is suggested that experiments with the intact system are necessary to elucidate the *in vivo* mode of action of the membrane proteins.

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